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Effect of Combined Oral Contraceptives on Insulin Clearance in Lean and Obese Pre-menopausal Women

Vidya Moorthy
Virginia Commonwealth University

School of Pharmacy
Virginia Commonwealth University

This is to certify that the thesis prepared by Vidya Moorthy entitled 'Effect of Combined Oral Contraceptives on Insulin Clearance in Lean and Obese Pre-Menopausal Women' has been approved by her committee as satisfactory completion of the thesis requirement for the degree of Master of Science in Pharmaceutical Sciences

Dr. Kai I.Cheang, Associate Professor, School of Pharmacy

Dr. Mary Jayne Kennedy, Assistant Professor, School of Pharmacy

Dr. Jessica Ketchum, Assistant Professor, School of Medicine

Dr. Donald F. Brophy, Chair, Department of Pharmacotherapy & Outcomes Science, School of Pharmacy

Dr. Susanna Wu.Pong, Director of the Pharmaceutical Science Graduate Program, School of Pharmacy

Dr. F. Douglas Boudinot, Dean of the School of Graduate Studies

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EFFECT OF COMBINED ORAL CONTRACEPTIVES ON INSULIN CLEARANCE IN LEAN AND OBESE PRE-MENOPAUSAL WOMEN

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Pharmaceutical Sciences at Virginia Commonwealth University

by

VIDYA MOORTHY

Bachelor of Pharmacy, Sri Ramachandra Medical College and Research Institute, India, 2008

Director: DR. KAI I. CHEANG, PHARM.D., M.S

ASSOCIATE PROFESSOR, DEAPRTMENT OF PHARMACOTHERAPY AND OUTCOMES SCIENCE

Virginia Commonwealth University

Richmond, Virginia

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Abstract

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Major Director: Dr. Kai I. Cheang, Pharm.D. M.S

Associate Professor, Department of Pharmacotherapy and Outcome Sciences

Introduction: Obese women are predisposed to greater risks of insulin resistance and compensatory hyperinsulinemia. Likewise, African-Americans, appear to be inherently insulin resistant and hyperinsulinemic even after controlling for obesity. Hyperinsulinemia has been attributed to insulin resistance and a compensatory insulin hyper-secretion by the pancreas, as well as decreased insulin clearance, notably in obesity. Pharmacological agents that may worsen insulin resistance/hyperinsulinemia in obese women is of clinical relevance. Previous data from our group suggested that combined oral contraceptives (COCs) may worsen insulin sensitivity particularly in obese

women, but limited information on insulin clearance is available in obese women or African-American women.

Objective: The objective of the study is to evaluate and compare the effect of a COC containing ethinyl estradiol and norgestimate on insulin clearance among lean and obese pre-menopausal women and among African-American obese vs. non African-American obese women.

Method: Plasma insulin clearance was calculated from plasma insulin concentrations, following frequently sampled intravenous glucose tolerance test. Changes in insulin clearance, during six months of COC use were analyzed by repeated measures analysis.

Result: Six months of COC use showed no significant change in insulin clearance in all women (p=0.3713). Furthermore, there were no divergent effects on insulin clearance among lean (n=13) and obese (n=14) women (p=0.6703) and among African-American obese (n=7) and non African-American obese (n=7) women (p=0.0957). Changes in insulin clearance, following six months of COC administration was found to be positively correlated with changes in insulin sensitivity (r=0.385, p=0.0099) and negatively correlated with changes in acute insulin response to glucose (r=-0.432, p=0.0034).

Discussion: In the present study, COC administration did not show any differential effect on insulin clearance in lean vs. obese women. Future studies evaluating the effects of hormonal agents on insulin-glucose dynamics may focus on mechanisms of hormone-mediated insulin resistance and compensatory hyperinsulinemia rather than insulin clearance.

1. INTRODUCTION

Obesity, defined as body mass index (BMI) $\geq$ 30 kg/m^2 has assumed epidemic proportion, with over 32% of the adult population in the United States found to be obese (1). Sixty percent of adult women in particular are found to be overweight (BMI $\geq$ 25 kg/m^2) (2).

Obesity raises the risk of type 2 diabetes mellitus, dyslipidemia, and coronary heart disease. Interestingly, 80% of the subjects with type 2 diabetes mellitus are obese and insulin resistant (3).

The major premise underpinning type 2 diabetes is insulin resistance (impaired insulin-mediated glucose uptake). Obesity has a significant role to play in the pathophysiology of insulin resistance (4) and consequent hyperinsulinemia (5). Obesity may accelerate insulin response by increasing β cell induced insulin secretion and decreasing insulin clearance, especially in insulin resistant individuals (6). Hyperinsulinemia is found to be a strong predictor for insulin resistance syndrome, type 2 diabetes mellitus, hypertension, coronary artery disease, stroke and even cancer (4;7;8). Thus, worsening of insulin resistance and consequent hyperinsulinemia in obese women is of immense clinical relevance.

Combined Oral Contraceptives (COCs) are the leading form of contraception in the United States (9) with over 11.6 million women receiving a prescription for it (10). Although COCs in

general do not worsen glucose tolerance (11), most studies evaluating the effect of COC on glucose metabolism have been performed in lean healthy women.

COC may also alter insulin clearance and lead to hyperinsulinemia. One study suggested the possible role of progestin (norethindrone) in reducing insulin clearance, consequently leading to hyperinsulinemia (12). These findings were replicated in another study, which demonstrated significant decrease in insulin elimination during COC use containing norethindrone and desogestrel (13). Both studies were conducted in healthy lean women.

Our research group has previously reported that insulin sensitivity is altered differentially depending on obesity status (14). Specifically, insulin sensitivity worsened with COC more so in obese women as compared with lean women. However, whether obesity status also affects COC's alteration of insulin clearance is unknown.

Hyperinsulinemia and abnormalities in hepatic insulin extraction has also been documented in ethnic groups with severe insulin resistance (15). Pathophysiologically, several studies have documented the greater prevalence of insulin resistance in the African-American population (16) in comparison to Caucasians. Studies have shown that the African-American group is inherently insulin resistant; displaying enhanced acute insulin response to glucose (AIRg) (17) and decreased insulin clearance in comparison to their Caucasian counterparts (18-21). Thus, a differential effect of COC on insulin clearance is also possible across ethnicity.

Hence, in the present study, we compared the effect of a low-dose cyclic COC containing ethinyl estradiol 35mcg and norgestimate 0.18/0.215/0.25 mg on insulin clearance in lean and obese pre-menopausal women, after six months of COC use. In addition, we also performed a pilot analysis to evaluate the effect of insulin clearance in African-American obese women and non African-American obese women. We also explored the relationship between changes in insulin clearance and changes in glucose-insulin dynamics following six months of COC use.

Specific Aims

We hypothesize that exogenous administration of a low-dose COC is likely to decrease insulin clearance, more so in obese as compared to lean pre-menopausal women, and more so in African-American women as compared with Caucasian women. We tested our objective through the following specific aims:

Specific Aim 1:

Test the effect of a low-dose COC (containing ethinyl estradiol and norgestimate) on insulin clearance after six months of use in all pre-menopausal women.

Specific Aim 2:

Compare the effect of a low-dose COC (containing ethinyl estradiol and norgestimate) on insulin clearance after six months of use in lean and obese pre-menopausal women.

Specific Aim 3:

Perform a preliminary analysis to determine the effect of a low-dose COC (containing ethinyl estradiol and norgestimate) on insulin clearance after six months of use in African-American obese and non African-American obese pre-menopausal women.

Specific Aim 4:

To explore the relationship between changes in insulin clearance and changes in glucose-insulin dynamics, following six months of COC use.

2. BACKGROUND

Obesity and Hyperinsulinemia

The prevalence of obesity has doubled from 15% to 30% in the past three decades (2). In the United States, more than 60% of adult women are overweight (BMI $\geq$ 25 kg/m^2). Obesity has been associated with conventional cardiovascular risk factors along with increase in inflammatory markers [high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α)], non-esterified fatty acids (NEFA), and coronary artery endothelial dysfunction (2). Obese women are pre-disposed to greater risks of type 2 diabetes mellitus and other cardiovascular complications.

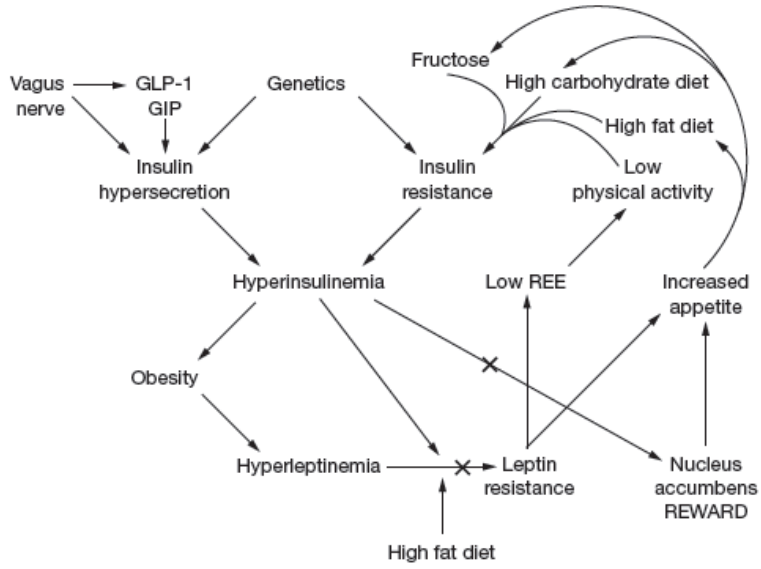
Obesity and insulin resistance/hyperinsulinemia are known risk factors for type 2 diabetes mellitus, cardiovascular diseases and hypertension (22). Also, obesity has been associated with insulin resistance and compensatory hyperinsulinemia (23).

A number of putative mechanisms that underlie the temporal relationship between obesity and insulin resistance/hyperinsulinemia have been suggested. Some studies suggest that obesity precedes the clinical expression of insulin resistance/hyperinsulinemia (22). It is hypothesized that increase in body fat (esp. visceral fat) mobilizes the circulation of free fatty acids (FFAs) in portal vein circulation, thereby reducing the hepatic insulin clearance, thus contributing to peripheral hyperinsulinemia (24). Utilization of excess FFAs by muscles, at the expense of glucose may contribute to insulin resistance in obesity (25). Similarly, increased levels of NEFA,

as observed in obesity, also contributes to insulin resistance as a result of competition with glucose for substrate oxidation (26). Increased NEFA levels results in increased intracellular content of fatty acid metabolites like diacylglycerol (DAG), fatty acyl-coenzyme, and ceramide, which results in phosphorylation of insulin receptor substrate 1 and 2 (IRS1 and IRS2), leading to decreased activation of phosphatidyl inositol-3-OH kinase [PI(3)K]. Decreased signalling of PI(3)K induces hepatic gluconeogenesis and insulin resistance (26). A post-receptor defect has also been postulated with obesity. With increase in the adipose cell size, reduction in the insulin effect on glucose oxidation has been reported owing to decrease in the number of insulin receptors (24). Further, increased secretion of mediators of inflammation like IL-6, CRP, TNF-α by adipose tissue in obesity have also been linked with insulin resistance (22;26;27). Also, levels of adiponectin, an insulin-sensitizer, stimulating fatty acid oxidation is known to be lowered in obesity (26).

Conversely, hyperinsulinemia may also contribute to obesity. Figure 1 shows an algorithm, describing the role of hyperinsulinemia in obesity. It is hypothesized that hyperinsulinemia interferes with leptin signal transduction in the hypothalamus, thereby promoting leptin resistance (28). Thus, the ability of leptin to stimulate α-melanocyte stimulating hormone and inhibit neuropeptide γ is hindered. This results in a decrease in resting energy expenditure (REE) and increase in appetite, promoting weight gain. In addition, hyperinsulinemia prevents dopamine reuptake at the nucleus accumbens, which promotes increased calorie intake (28).

Figure 1: Role of hyperinsulinemia in obesity



Abbreviations: GLP-1- Glucagon like peptide 1; GIP- Gastric inhibitory polypeptide

Various factors contribute to hyperinsulinemia, including vagus nerve induced insulin hyper-secretion, insulin resistance, and decreased insulin clearance (28). It is believed that hyperinsulinemia, insulin resistance and glucose stimulated insulin release are intertwined biologically (8). Hyperinsulinemia could result as a compensatory response to decreased insulin sensitivity. β-cells adapt to a chronic state of insulin resistance by secreting more insulin in response to a given plasma glucose levels (29). At the same time, hyperinsulinemia might itself perpetuate insulin resistance. Continuous exposure to insulin causes a reduction in the insulin receptor exposed on the cell surface, promoting internalization, followed by degradation (8).

Savage et al conducted a study to evaluate if hyperinsulinemia associated with obesity was a result of reduced hepatic extraction or hyper-secretion of insulin by the pancreas. In a study

conducted in normoglycemic, 10 Pima Indians and 10 Caucasians with varying degrees of obesity, the researchers found that the molar ratio of C-peptide to insulin was not significantly correlated with varying degree of obesity (r=0.08) (30). However, insulin and C-peptide concentrations were significantly correlated with degrees of obesity, with elevated levels of C-peptide and insulin in obese subjects. These results indicate that hyperinsulinemia in obese subjects is a result of pancreatic hyper-secretion. Additionally, obesity may also affect insulin clearance. In a study by Meistas et al, total metabolic clearance of insulin was 33% lower in obese subjects than non-obese subjects (31). Hyperinsulinemia has also been closely linked to decreased hepatic insulin degradation in subjects with cirrhosis and liver dysfunction (32). These results underscore the fact that decreased hepatic insulin extraction could be a possible reason for hyperinsulinemia in obesity.

African-American Race and Hyperinsulinemia

Rising rates of obesity among African-Americans in comparison to Caucasians (33;34), along with a confluence of lifestyle-environmental and genetic factors, may have contributed to the greater prevalence of type 2 diabetes and concomitant cardiovascular complications in the African-American population (16). African-American women in particular showed 2 fold greater prevalence of type 2 diabetes in comparison to Caucasian women (35;36).

However, lifestyle factors alone do not explain the disproportionately increased insulin resistance in African-Americans. Epidemiological studies have shown that African-Americans are significantly more insulin resistant and hyperinsulinemic than their Caucasian counterparts, even

after adjusting for body fat (20;35-38). In the Bogalusa Heart Study of 377 children (5-17 years), in comparison to Caucasian children, African-American children were found to have higher insulin response during oral glucose tolerance test (OGTT) and greater insulin to glucose ratio, suggestive of inherent insulin resistance (17). Hyperinsulinemia in African-Americans have been explained by hyper-secretion of insulin, as a compensatory response to decreased insulin sensitivity, and also by decreased insulin clearance (18-21;35;36;38), notably in obesity (36). Both lean and obese African-American pre-menopausal women were found to have significantly higher AIRg in comparison to their weight-matched Caucasian peers (36;37). In a study by Albu et al, a 163% significant increase in AIRg was noted in healthy African-American pre-menopausal women when compared to Caucasian women (39). In another study, 14% lower insulin clearance and 63% higher first phase insulin secretion than Caucasian adolescents, accounted for hyperinsulinemia among African-American adolescents (38). One possible reason for decreased insulin clearance could be because of lower liver mass among African-Americans (20). In a study conducted in 22 healthy, pre-pubertal African-American and Caucasian children, African-American children were found to have 15% lower insulin clearance when compared with Caucasian children, even after adjusting for adiposity. Similarly, these results were replicated even in African-American adults (15;36).

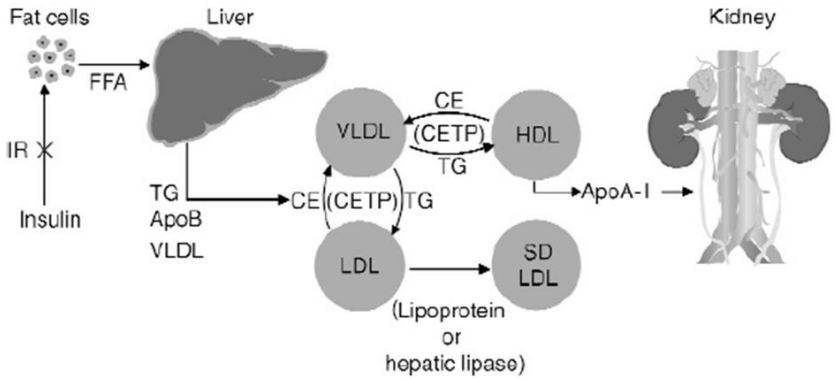
Clinical Consequence of Hyperinsulinemia

Clinical consequence of insulin resistance and compensatory hyperinsulinemia are increasingly appreciated for their role in type 2 diabetes mellitus and associated macro-vascular complications.

Insulin resistance and consequent hyperinsulinemia precedes the clinical expression of type 2 diabetes mellitus. Subjects with diabetes are 2-4 times more likely to have heart disease (40). Epidemiologic data have suggested that insulin resistance and consequent hyperinsulinemia is a risk factor for cardiovascular disease. Compared to normoinsulinemic, non-obese subjects; hyperinsulinemic, non-obese subjects with impaired glucose tolerance were associated with a cluster of cardiovascular risk factors such as elevated triglycerides, low levels of high density lipoprotein (HDL) and elevated systolic blood pressure (41). In the San Antonio Heart Study, hyperinsulinemia predicted the development of type 2 diabetes, low levels of HDL, high triglyceride levels, and hypertension over 8 years of follow-up (40;42). Additionally, in a meta-analysis of 12 prospective based studies, insulin was found to be a positive indicator of cardiovascular disease, particularly in middle aged adults (40). The Quebec Cardiovascular Study and the Insulin Resistance Atherosclerotic Study (IRAS) also provides a strong association between insulin resistance/hyperinsulinemia and development of cardiovascular disease (40;42). In the IRAS study, a significant association was reported between insulin resistance and intima media thickness of the carotid artery.

Hyperinsulinemia has the potential to raise or maintain the blood pressure by promoting renal sodium absorption and stimulating the sympathetic nervous system (43). Also, the vasodilatatory action of insulin, owing to endothelial nitric oxide release, is reduced in obese subjects (43). Insulin resistance and consequent hyperinsulinemia is a strong predictor of coronary artery disease (4). Insulin resistance results in increased release of FFA into the circulation (7). Flux of FFA in the liver stimulates the secretion of very low density lipoprotein (VLDL), leading to hypertriglyceridemia. VLDL stimulates the exchange of cholesteryl esters (CE) from HDL and low density lipoprotein (LDL) for VLDL triglycerides (VLDL TG). Apolipoprotein A-I that dissociates from TG-enriched HDL, reduces the availability of HDL for reverse cholesterol transport. Also, TG-enriched LDL undergoes lipolysis to give rise to small, dense LDL. Culmination of low levels of HDL and the presence of small, dense LDL contribute to cardiovascular disease (7). Figure 2 provides a simplified model linking insulin resistance and cardiovascular disease.

Figure 2: Model linking insulin resistance and cardiovascular disease



Hyperinsulinemia may also directly inhibit fibrinolysis in obese, insulin resistant individuals (7). Visceral obesity is associated with increased levels of plasminogen activator inhibitor-I (PAI-I),

which complexes with plasminogen and eliminates its fibrinolytic activity (4). Hyper-coagulability and impaired fibrinolysis in insulin resistant individuals add to the pathological basis for increased cardiovascular events (7).

Combined Oral Contraceptives and Glucose-Insulin Metabolism

COCs are the most common form of contraception, with over 100 million women using it worldwide (10). In the US alone, 11.6 million women are COC users (10). In fact, 4 of the top 200 prescribed drugs in the US comprise of COCs.

Studies have substantiated the ability of COC to impair glucose tolerance within six months of use (12;13;44-46). In one of the earlier studies, Doar et al studied the effects of COC use and obesity on plasma glucose and pyruvate levels. In comparison to non-obese subjects, obese subjects were found to have significantly higher blood glucose and pyruvate levels (47). However, it was a cross-sectional study, providing little information on different indices of glucose metabolism.

Exogenous estrogens may have different effects on carbohydrate metabolism based on doses used (48). In comparison to higher doses, lower dose COC are known to produce fewer side effects (49). Administration of exogenous low concentration (10X) of 17 β estradiol (dose=0.326µg/day) in ovariectomized rats was found to up-regulate IRS-1, subsequently increasing the insulin sensitivity in muscle and adipose tissue (50). However, high concentration

(100X) of 17 β estradiol (dose=0.326µg/day) was found to produce an opposite effect (50). High-dose COC (ethinyl estradiol- EE >50 µg + progestin) use in particular resulted in the development of impaired glucose tolerance in 15.4% of current COC users (n=354) versus 6.3% of non-COC users (n=1732) (44). In women with diabetes mellitus, administration of high-dose COC resulted in further worsening of glucose metabolism in 73% of the users (44). In women with previous gestational diabetes, high-dose COC (0.08 mg mestranol) use resulted in the development of impaired glucose tolerance in 2 of 12 subjects, along with deterioration in 4 of 12 subjects in a period of 2 weeks. Integrated insulin response to glucose, though delayed, was also found to increase by 2 fold (51). Additionally, in a cross-sectional study conducted in women on COC containing 0.1mg mestranol + 2mg norethindrone (high-dose) for a period of 9.5 years, 12 out of 31 subjects were found to have abnormal glucose tolerance (46). Marginally reducing the dose and duration of COC use (sequential type COC containing 0.08mg mestranol + 2mg chlormadinone acetate for a period of 6.5 years) resulted in only 1 out of 31 women with abnormal glucose tolerance (46). These results substantiate the deleterious effects of high-dose COC on glucose metabolism. Thus, a transition from high-dose to low-dose COC use was made in clinical practice. A recent Cochrane analysis suggested that low-dose COC (EE $\leq$ 35 µg) may have limited effects on glucose metabolism in normal weight women (11). However, a few studies have shown that even low-dose COC, with estrane (norethindrone) and gonane (levonorgestrel, norgestrel) progestins, are associated with impaired glucose homeostasis (44;45). In a study by Wynn, 210 women who initiated COC (EE 30 µg + levonorgestrel 150 µg) were prospectively followed for a period of 3 years. At the end of 3 months, 13% of the subjects were found to be glucose intolerant. While at 15th, 25th and 37th months, the number of glucose intolerant subjects were found to increase to 10%, 20% and 30% respectively (46). Similarly, in

a cross-sectional study, low-dose Lovral® (EE 30 µg + norgestrel) users also showed lowered insulin sensitivity and glucose effectiveness (ability of glucose to promote its uptake at basal insulin levels) in comparison to control users (never used or discontinued COC use in the past 24 months) (45). Hence, although the recent Cochrane analysis showed that low-dose COC use had little effect on glucose metabolism in healthy lean women with no known risk of diabetes (11), evidence exist that the risk is present. Importantly, we have limited information regarding obese women (11).

Effect of low-dose COC on glucose metabolism also depends on the type of progestins. Progestins have been shown to antagonize the effect of insulin on glucose metabolism in adipose tissue and skeletal muscle, by bringing about a decrease in the target tissue insulin receptor number and affinity (49). Gonane progestins (levonorgestrel, norgestrel) in particular may elevate blood glucose and insulin levels (11;44). On the other hand, newer progestins such as desogestrel and drospirenone were found to have a limited effect on carbohydrate metabolism, when used for 1 year (44;52). COC's progestin component's effects on carbohydrate metabolism has been attributed to their androgenic activity (46;48). Low androgenic hormonal contraceptives (e.g. containing medroxy progesterone acetate) were associated with reduced risk of gestational diabetes mellitus (Odds Ratio=0.84, 95% CI 0.58-1.22), in comparison to high androgenic contraceptives like levonorgestrel (Odds Ratio=1.43, 95% CI 0.92-2.22) (53). In a study comparing the effects of COC with the progestin drospirenone (17-α spirolactone derivative, with anti-androgenic property) and desogestrel on glucose metabolism, no significant changes in fasting glucose and insulin levels were observed at the end of cycle 6 and 13, in both the treatment groups. The mean area under the curve-AUC (0-3 hr) for glucose moderately increased

by 28.7 mg/dL*h in the drospirenone group and by 22.2 mg/dL*h in the desogestrel group. Modest increase in mean insulin levels was also observed. But none of these effects were significant (52). Norgestimate, the proposed progestin in our study has little adverse effects on carbohydrate metabolism. In a study by Burkman et al, no significant changes in fasting blood glucose, insulin or glycosylated hemoglobin levels were observed at the end of 2 years of COC use containing norgestimate (54).

Studies have also suggested that the progestin component may prolong insulin half life and decrease insulin clearance (12;55;56). In a study evaluating the effect of COC (combined formulations of 30-40 µg EE with triphasic regimen of levonorgestrel and monophasic regimen of desogestrel and norethindrone) on glucose metabolism in healthy Caucasian women, the researchers found that the desogestrel combination significantly decreased the insulin elimination constant in comparison to non-COC users (13). The mean insulin half-life was found to be 5.06 minutes in non-COC users and 6.48 minutes in desogestrel combination users (13). Similar effects have also been demonstrated by norethindrone-type preparations. Addition of norethindrone 1mg to ethinyl estradiol was found to significantly decrease the rate constant for insulin disappearance, when compared to ethinyl estradiol alone (9.97 ± 0.25%/min vs. 9.39 ± 0.22%/min, $p < 0.03$) (12). These results suggest the possibility that the progestin component may decrease insulin clearance, and thereby prolonging its circulation.

Knowledge Gap

Previous studies have suggested that hyperinsulinemia could be a result of compensatory increased secretion of insulin, due to insulin resistance or due to decreased insulin clearance, notably in obesity (35;36;57). Our research group has previously conducted a study to determine if there was a differential effect of COC on insulin sensitivity, depending on obesity status. The study found divergent effects of COC on insulin sensitivity in lean vs. obese women, with obese women displaying lowered insulin sensitivity (14). Six months of COC use worsened insulin sensitivity in obese women, with no significant change in AIRg. If insulin sensitivity and AIRg are related by a hyperbolic curve (58), then reduction in insulin sensitivity in obese subjects would have been compensated by increased AIRg. However, in the aforementioned study by our research group, no change in AIRg was observed with COC use. This postulated the possibility of decreased insulin clearance, contributing to hyperinsulinemia, with COC use. Hence, the objective of the study was to determine if insulin clearance is different in obese vs. lean pre-menopausal women after COC use.

Numerous studies have suggested that African-Americans have a greater degree of obesity and are predisposed to insulin resistance/hyperinsulinemia and consequent type 2 diabetes mellitus and cardiovascular complications, in comparison to their Caucasian counterparts (18-20;35;36;38;39;59;60). Hence, we also tested the effect of COC on insulin clearance in African-American obese vs. non African-American obese pre-menopausal women.

Significance

Obese women are at a greater risk of insulin resistance and hyperinsulinemia (23;25-27). Hyperinsulinemia has been associated with insulin resistance syndrome, hypertension, coronary artery disease, and coagulation abnormalities (4). Hyperinsulinemia can result from insulin resistance or decreased insulin clearance. Although COC's effect on glucose metabolism and insulin resistance in lean women are well known, data on obese women are sparse. This is a critical knowledge gap, as obese women are already at a greater risk of insulin resistance and hyperinsulinemia, and further worsening of this condition is of clinical relevance to these women. The specific objective of this study is to evaluate the effect of a low-dose COC (containing ethinyl estradiol and norgestimate) on insulin clearance in lean and obese pre-menopausal women. Furthermore, we tested if the difference in insulin clearance differed across African-American obese and non African-American obese women, owing to greater prevalence of obesity, insulin resistance/hyperinsulinemia in this population (34-36).

The proposed study is innovative because no study to date has prospectively compared the effect of COC on insulin clearance in lean and obese pre-menopausal women. This contribution is significant because it is a step towards a continuum of research in deciphering the processes underlying impairment of glucose metabolism in obese women upon COC use.

3. METHODS

Study Design

Our analysis of insulin clearance data was performed using data from a previously conducted prospective single center trial, evaluating 13 lean (control group) and 14 obese pre-menopausal women over a period of six months (14). In this study, we evaluated the effect of a low-dose COC (containing ethinyl estradiol and norgestimate) on glucose-insulin homeostasis. Details of this previous study are provided below along with the methodology used for the estimation of insulin clearance in the current study.

Intervention

COC containing ethinyl estradiol 35 mcg and norgestimate 0.18/0.215/0.25 mg (Ortho Tri-Cyclen®, Ortho-McNeil Pharmaceutical). This COC is one of the most widely prescribed COC in the United States (9).

Study Population

Participants were deemed eligible in the study provided they met the following **inclusion criteria**: (i) Pre-menopausal women, 18 to 40 years of age, (ii) lean women with BMI < 25 kg/m^2 and obese women with BMI ≥ 30 kg/m^2, (iii) with no COC use within past three months of the study, (iv) expressing willingness and providing an informed consent to take part in the study, (v) and expressing their ability to comply with the study requirements. The following were the **exclusion criteria**: (i) presence of diabetes mellitus, assessed by OGTT (fasting glucose ≥

126mg/dl or a 2 hour glucose ≥ 200mg/dl), (ii) systolic blood pressure > 160mmHg or diastolic blood pressure > 100mmHg, (iii) presence of any pulmonary, cardiac (history of thromboembolism, myocardial infarction, cerebrovascular accident, vascular disease or known coagulopathy), renal, hepatic, neurological (including migraine, headaches), psychiatric, infectious and malignant diseases, (iv) use of any hypoglycemic, gluco-corticoids, anti-androgens (e.g. spironolactone, flutamide), anti-hypertensive, lipid lowering agents that are known to affect the glucose metabolism, (v) prolonged immobilization, or major surgery within past six months, (vi) use of any investigational drugs within three months, (vii) pregnant or lactating women, (viii) anemic women (hematocrit < 33%), (viii) smoker ≥ 20 cigarettes/day, and (ix) subjects actively attempting weight loss.

Recruitment

Subjects were recruited from the student health clinics and obstetrics/gynecology centers at Virginia Commonwealth University Medical center via poster, fliers in and around the campus. The study was conducted at the General Clinical Research Center (GCRC).

Potential subjects were then enrolled into the study only after obtaining an informed consent. Participants visited the GCRC for study procedures at baseline, and after three and six months of COC administration.

The study was approved by the Institutional Review Board (IRB) at Virginia Commonwealth University.

Study Protocol

Recruited participants presented to the GCRC after a 12-hour overnight fast during the follicular phase of their menstrual cycle (confirmed by a serum progesterone level of <2ng/ml). On day 1, an OGTT with 75 gm of glucose was performed. On day 2, participants underwent the modified frequently sampled intravenous glucose tolerance test (FSIVGTT), as described by Bergman (61). At time 0, 300mg/kg glucose solution was administered intravenously as a bolus over 1 minute, followed by 0.03U/kg insulin infusion at 20 minutes. Blood was then drawn at -15, -5, 0, 2, 3, 4, 5, 6, 8,10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 minutes (61;62).

Participants were then started on a COC [Ortho Tri Cyclen; containing ethinyl estradiol 35mcg and norgestimate0.18/ 0.215/0.25 mg for a period of six months]. All participants were instructed to take the active pill everyday for 21 days, followed by a 7 day pill free period. Participants were also instructed to maintain their normal dietary and physical activity during the six month period.

Follow up

A first follow up was scheduled three months from COC use, between 5th and 7th day of the hormone-free week, to minimize the effect by progestins on insulin kinetics. A repeated assessment of the 2-hr OGTT was performed. A second follow up visit was scheduled six months from baseline, between 5th and 7th day of the hormone-free week. On days 1 and 2 of the six month follow up visit, study procedures were the same as days 1 and 2 of the baseline visit.

Methodology

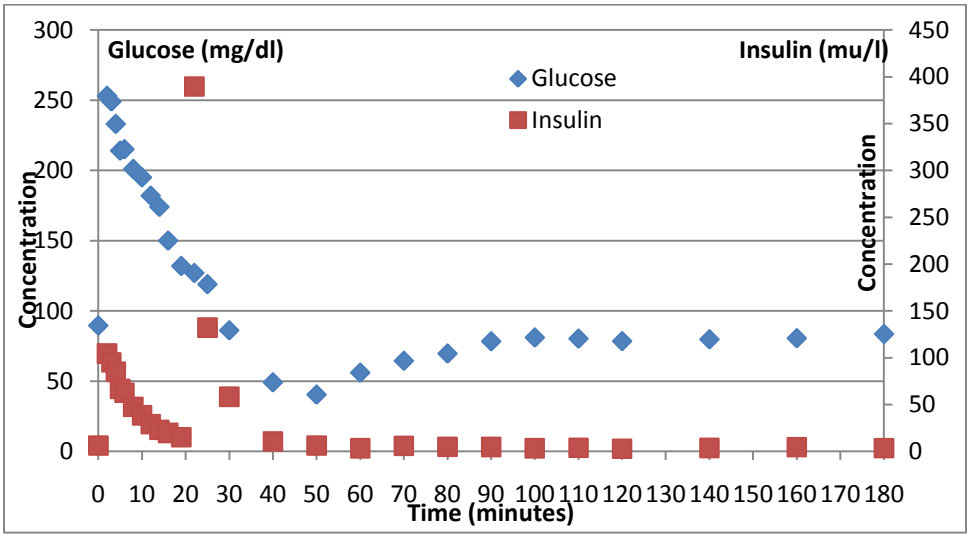
All samples to be analyzed were stored at -70°C. Glucose was analyzed by glucose oxidase methodology (YSI 2300 Stat Plus Glucose Analyzer, Yellow Spring Instruments (YSI), Yellow Springs, OH), while plasma insulin was analyzed using ELISA (ALPCO Diagnostics, Salem, NH). All samples were analyzed in duplicates.

Measurement of insulin clearance:

Plasma insulin clearance was estimated from plasma insulin measurements from FSIVGTT, taken after exogenous administration of insulin bolus infusion, assuming a single compartment model.

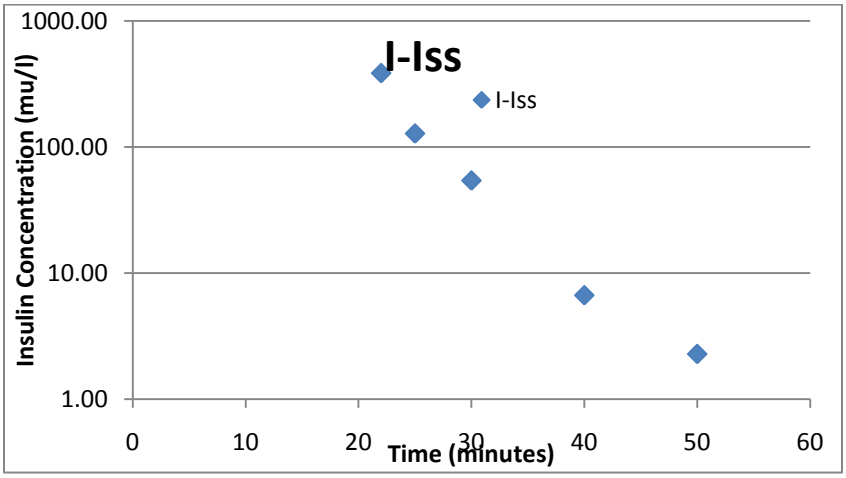
Plasma insulin concentration declines following administration of intravenous infusion of insulin at 20 minutes. Figure 3 provides a description of how plasma insulin concentration diminishes following intravenous insulin administration at 20 minutes. However, plasma insulin level stabilizes and attains a steady state around 50-60 minutes.

Figure 3: Plasma insulin-glucose concentrations, following FSIVGTT



Steady state insulin concentration (Iss) was calculated by taking an average of the plasma insulin levels after it stabilizes. The difference between insulin measurements (from peak insulin concentration following insulin bolus infusion to steady state insulin concentration) is I-Iss. Figure 4 provides a graph of I-Iss in a woman with a BMI of 24kg/m^2.

Figure 4: I-Iss



Insulin elimination constant (Ke, min^{-1}) was determined by the negative slope of the regression of the logarithm (I-Iss) against time (i.e. time from peak insulin concentration following insulin infusion to steady state insulin concentration) multiplied by 2.303.

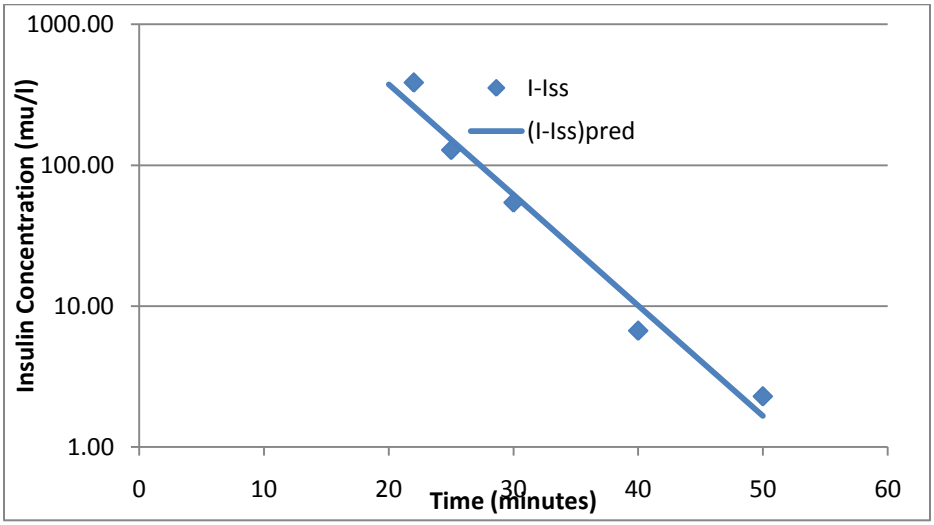
Volume of distribution (Vd, L) was calculated using the formula:

$Vd = dose/C_0$;

Where dose was the amount of insulin infusion per kg body weight and C_0 was the predicted plasma insulin concentration (I-Iss) at 20 minutes.

Figure 5 provides a graph of both (I-Iss) and predicted (I-Iss) for the same woman as described above.

Figure 5: (I-Iss) and predicted (I-Iss)



Finally, total insulin clearance (CL_{tot}, L/min) was calculated using the formula (63):

$CL_{tot} = Vd * Ke$

Measurement of glucose-insulin dynamics:

Glucose-insulin dynamics was calculated from modified FSIVGTT, using the Minimal Model Identification Software (MINIMOD), version 6.02, Los Angeles, CA) (64). The aforementioned software is a robust, accurate, reproducible and user friendly software for measuring the glucose-insulin dynamics. Insulin sensitivity measured by this method indicates the net capacity of insulin to promote peripheral and hepatic glucose uptake and also inhibit endogenous glucose production. Insulin sensitivity index (Si) is calculated using the formula; $Si=P_3/P_2$ (where P_3 is a parameter describing the levels of circulating insulin in interstitial fluid and P_2 is a parameter describing the removal rate of insulin from interstitial fluid) (61;64). The minimal model also provides estimation of glucose effectiveness (Sg), AIRg (area under the plasma insulin curve between 0 and 10 minutes, which is a measure of first phase insulin secretion), and disposition index (DI, which is Si * AIRg, composite measure of insulin secretion and action) (64).

Insulin and glucose incremental area-under-the-curve (AUC) upon OGTT was analyzed by the trapezoidal rule after subtracting baseline values. Glucose-insulin values from OGTT was also used to calculate the Matsuda insulin sensitivity index (65), using the following formula: [10,000/ √ {(fasting plasma glucose * fasting plasma insulin) * (mean OGTT glucose concentration * mean OGTT insulin concentration)}]. Homeostasis model assessment or HOMA

uses a computer solved model to estimate insulin sensitivity and β cell function using the basal steady state plasma glucose and insulin levels, given by the formula:

*HOMA-Insulin Sensitivity Index=405/ [Fasting glucose (mg/dl) * Fasting insulin]* (61;66).

Data Analyses

JMP 8 statistical software (SAS Institute Inc., Cary, NC) was used to perform all the analysis using a significance level of 0.05. The primary outcome of interest was the mean change in insulin clearance from baseline after six months of COC administration, among obese vs. lean pre-menopausal women. Additionally, mean change in insulin clearance was also assessed among African-American obese vs. non African-American obese pre-menopausal women. We also looked into the overall effect of COC use on insulin clearance in all women regardless of baseline obesity status after six months of COC administration. Relationship between changes in insulin clearance with six months of COC use and changes in glucose-insulin indices was also evaluated.

Normality distribution was confirmed. All continuous variables were described using mean and standard deviation, while categorical variables were described using counts and proportion. Baseline comparisons between lean and obese women were performed using students' t-test in case of equal variance, while Welch ANOVA was used if unequal variance was observed.

Our primary research question, i.e. mean change in insulin clearance from baseline after six months of COC use, among lean and obese pre-menopausal women was tested by repeated measure analysis. The model consisted of insulin clearance as the outcome variable; subject ID as a random effect, time trend, obesity status along with their interaction with time trend as fixed effects. Similarly, mean change in insulin clearance from baseline after six months of COC use in African-American obese and non African-American obese women was tested by repeated measure analysis. Change in insulin clearance from baseline to six months in all women regardless of obesity status was analyzed by paired t-test. Also, change in insulin clearance within each of the group (lean, obese, African-American obese and non African-American obese) was assessed by matched paired t-test.

Relationship between changes in insulin clearance and changes in glucose-insulin dynamics, following six months of COC use was evaluated by a simple correlation test.

4. RESULTS

Baseline Characteristics

A total of 48 pre-menopausal women provided their informed consent for the study. However, only 27 women completed the entire course of the trial, with 4 no-shows, 4 failing to meet the screening-eligibility criteria, 5 losses to follow up and 8 withdrawing from the study. Table 1 provides the distribution of body type and race in the analyzed (n=27) and the unanalyzed (n=21) group.

Table 1: Distribution of body type and race in the analyzed and unanalyzed group

PARAMETER		**GROUP**		
		Analyzed (n=27)	**Unanalyzed (n=21)**	**P-value**
Body Type	*Lean (BMI < 25kg/m^2)*	13 (48.15%)	2 (9.5%)	<0.0001*
	Obese (BMI ≥ 30kg/m^2)	14 (51.85%)	7 (33.33%)	0.0018*
	Information Unavailable	-	12 (57.14%)	-
Race	*African-American*	8 (29.63%)	4 (19.05%)	0.0185*
	Caucasian	17 (62.96%)	8 (38.1%)	0.0002*
	Hispanic	2 (7.4%)	0	-
	Asian	0	2 (9.52%)	-
	Information Unavailable	-	7 (33.33%)	-

*Indicates significant difference ($p<0.05$)

The 27 women included in the study consisted of lean (n=13) and obese (n=14) women (see Table 2). Seven of the 14 obese women were African-Americans, while the other seven were Caucasians (table 3).

Table 2: Proportion of lean and obese women

Group	N	Proportion
Lean	13	0.481
Obese	14	0.518

Table 3: Proportion of African-American obese women

Group	N	Proportion
Non African-American obese women	7	0.500
African-American obese women	7	0.500

At baseline, ages of the lean and obese women in the study were similar. As predicted, obese women had significantly higher BMI, systolic-diastolic blood pressure (BP), waist circumference and waist-hip-ratio. Baseline demographic characteristics of the study subjects are summarized in table 4.

Table 4: Baseline demographic characteristics of study subjects

Parameter	Lean women (n=13)	Obese women (n=14)	P value
Age (yrs)	21.23 ± 2.31	22.5 ± 5.24	0.4300
BMI (Kg/m^2)	20.99 ± 1.57	36.93 ± 6.75	<0.0001 *
Systolic BP (mmHg)	105.97 ± 9.31	121.71 ± 13.83	0.0020 *
Diastolic BP (mmHg)	68.28 ± 3.86	74.05 ± 8.67	0.0364 *
Waist Circumference (cm)	67.85 ± 8.39	99.56 ± 13.95	<0.0001 *
Waist-Hip-Ratio	0.705 ± 0.08	0.787 ± 0.057	0.0051 *

*Indicates significant difference ($p<0.05$)

Mean ± standard deviation

Baseline comparison of glucose-insulin dynamics showed that obese women were less insulin sensitive than lean women. Using the minimal model, we found that obese women had significantly lower insulin sensitivity (difference= -3.28 units, S.E= 1.10, 95% CI= -5.56 to -

1.00) than lean women (t= -2.97, df= 24, p= 0.0066). Significantly lower insulin sensitivity in obese women was also demonstrated by HOMA (t= -3.245, df= 25, p= 0.0033) and Matsuda indices (t= -2.641, df= 25, p= 0.0149). The results are summarized in table 5.

Low insulin sensitivity in obese women was compensated by hyper-secretion of insulin by the pancreas. Obese women had significantly higher AIRg (t= 4.378, df= 18.295, p= 0.0004) in comparison to their lean counterparts. No significant difference in insulin clearance was observed between the two groups at baseline (table 7).

Table 5: Baseline comparison of glucose-insulin dynamics

Parameter	Lean women (n=13)	Obese women (n=14)	P value
	Baseline	**Baseline**	
AIRg[mu.L^{-1} .min]	324.84 ± 93.69	840.38 ± 92.36	0.0004 *
Si[min^{-1}/mu/L]	7.19 ± 0.823	3.85 ± 0.812	0.0066 *
Incremental AUC glucose	3080.6 ± 590.97	2732.4 ± 569.47	0.6622
Incremental AUC insulin	3905.9 ± 622.89	4400.84 ± 600.23	0.6151
Sg (1000.min^{-1})	0.037 ± 0.0058	0.028 ± 0.0058	0.4186
DI (AIRg.Si)	2161.35 ± 444.33	3126.74 ± 439.35	0.1352
ISI HOMA	1.56 ± 0.215	0.92 ± 0.207	0.0033 *
Matsuda index	11.74 ± 1.35	7.22 ± 1.3	0.0149 *

Least square mean ± standard error

*indicates significant

Specific Aim 1: Effect of COC on insulin clearance in all women

Six months of COC use did not have any significant effect on insulin clearance when all the 27 women were analyzed together. The results are summarized in table 6.

Table 6: Effect of COC on insulin clearance in all women

Insulin clearance (l/min)	**Baseline**	**6 months**	**Difference (6 months-baseline)**	**P value**
	1.11 ± 0.078	1.2 ± 0.078	0.09 ± 0.099 (*95% CI*= -0.115 to 0.296)	0.3713

Least square mean ± standard error

Specific Aim 2: Effect of COC use on insulin clearance in lean vs. obese women

No significant difference in insulin clearance was observed after six months of COC use, based on obesity status, as summarized in table 7. Within each of the group (i.e. lean and obese group), no significant difference in insulin clearance was observed with six months of COC use.

Even after adjusting the insulin clearance for body weight, no significant difference [$F_{(1, 25)} = 0.067$, $p=0.7978$] in insulin clearance was observed after six months of COC use between lean and obese women.

Table 7: Effect of COC on insulin clearance in lean vs. obese women

Parameter	Lean women (n=13)		Obese women (n=14)		P value (baseline comparison between groups)	P value (comparison of COC effects between groups)
	Baseline	6 months	Baseline	6 months		
Insulin clearance (l/min)	1.22 ± 0.113	1.27 ± 0.113	1.01 ± 0.109	1.15 ± 0.109	0.1368	0.6703
P value (paired t-test)	0.7893		0.2884			

Least square mean ± standard error

Specific Aim 3: Effect of COC on insulin clearance in African-American obese vs. non African-American obese women

At baseline, there was no significant difference in insulin clearance between African-American obese vs. non African-American obese women (Table 8). Six months of COC use resulted in no significant effect on insulin clearance between the two groups. No significant change in insulin clearance was also observed within each of African-American obese and non African-American obese women group.

Table 8: Effect of COC on insulin clearance in African-American obese vs. non African-American obese women

Parameter	African- American obese women (n=7)		Non-African-American obese women (n=7)		P value (baseline comparison between groups)	P value (comparison of COC effects between groups)
	Baseline	6 months	Baseline	6 months		
Insulin clearance (l/min)	0.963 ± 0.129	0.895 ± 0.129	1.07 ± 0.129	1.4 ± 0.129	0.5184	0.0957
P value (paired t-test)	0.2816		0.1706			

Least square mean ± standard error

Specific Aim 4: Relationship between changes in insulin clearance and changes in glucose-insulin dynamics with COC use (taking all the women into consideration)

Changes in insulin clearance with six months of COC was found to be significantly related to changes in AIRg, insulin sensitivity as measured by minimal model, incremental AUC glucose and glucose effectiveness (Table 9). Changes in insulin clearance was positively related with changes in insulin sensitivity (figure 6) and negatively related with changes in AIRg (figure 7). However, no significant relationship was observed between changes in insulin clearance and changes in incremental AUC glucose, disposition index, and insulin sensitivity measured by HOMA and Matsuda index.

Table 9: Relationship between changes in insulin clearance and changes in glucose-insulin dynamics after six months of COC use

	ΔInsulin clearance (l/min)	
Parameter	**Correlation (r)**	**P value**
ΔAIRg [mu.L^{-1} .min]	-0.432	0.0034 *
ΔSi [min^{-1}/mu/L]	0.385	0.0099 *
ΔIncremental AUC glucose	0.4257	0.0040 *
ΔIncremental AUC insulin	0.1298	0.401
ΔSg (1000.min^{-1})	0.484	0.0009 *
ΔDI (AIRg.Si)	-0.029	0.8503
ΔISI HOMA	0.225	0.1413
ΔMatsuda index	0.120	0.4363

*indicates significant

Figure 6: Scatter plot showing the relationship between changes in insulin clearance and changes in insulin sensitivity following six months of COC use

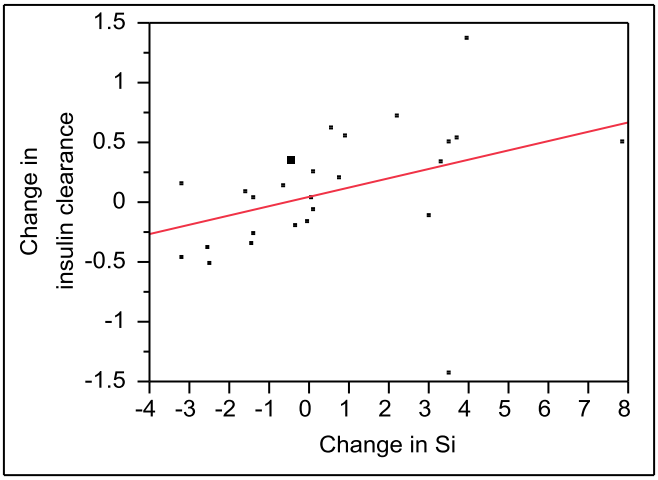
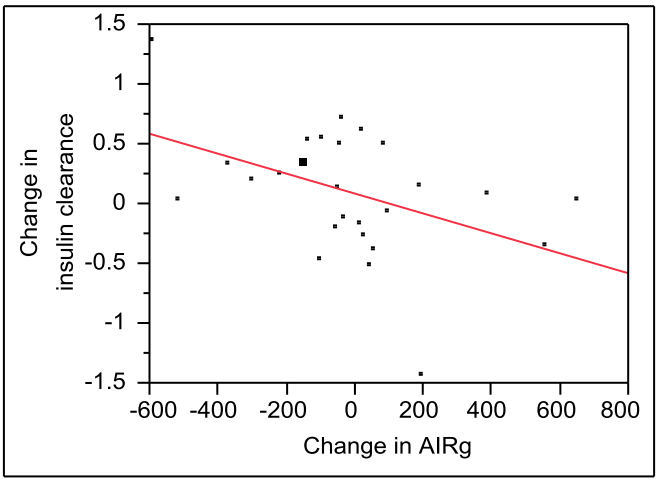


Figure 7: Scatter plot showing the relationship between changes in insulin clearance and changes in AIRg following six months of COC use



5. DISCUSSION

Obesity has been correlated with hyperinsulinemia, and this effect is attributed to both compensatory hyper-secretion of insulin by the pancreas secondary to insulin resistance and decreased insulin clearance. In a previous study, we have reported the effects of COC on insulin sensitivity in lean and obese women (14). Six months of COC use showed divergent effects on insulin sensitivity in lean vs. obese women, as measured by the minimal model ($p=0.0494$). These effects were also demonstrated by other measures of insulin sensitivity, such as the Matsuda index ($p=0.0227$) and ISI HOMA ($p=0.0128$). In the above mentioned study, following six months of COC administration, insulin sensitivity worsened in obese women and improved in lean women. No significant difference was observed between lean and obese women in changes in AIRg during six months of COC use (14). Considering the worsening of insulin sensitivity and no change in AIRg in obese women, we were interested to see if insulin clearance had a role to play in contributing to hyperinsulinemia. Hence, in this study, we set out to examine whether a commonly used COC affect insulin clearance, and whether effects on insulin clearance is different between lean and obese women.

Insulin clearance involves both first-pass hepatic elimination (30) and peripheral insulin uptake, internalization and degradation. At physiological concentration (10^{-9} M), insulin uptake is a receptor mediated process, while at non-physiological concentration, non-receptor mediated processes predominates (67). Liver contributes to about 50-70% of insulin degradation (29), while kidney is the main site for insulin clearance from the systemic circulation (67). Other sites

of insulin uptake and degradation comprises of pancreas, adrenal gland, testis, spleen, ovary, lung, heart, muscles, brain and fat.

In the present study, there was no differential effect on insulin clearance in lean vs. obese women, after six months of COC administration. One of the possible reasons for this could be because of our choice of progestin. We based our choice of COC (ethinyl estradiol 35 mcg and norgestimate 0.18/0.215/0.25 mg) used in the study on agents that are most commonly used. Although we reported a differential effect of norgestimate-containing COC on insulin sensitivity in lean vs. obese women in a previous study (14), other studies have shown a metabolic neutral effect. Norgestimate, a 3rd generation gonane progestin is known to have minimal metabolic effects (54;68-72). It also has a low androgenic profile (70). Use of a norgestimate-containing oral contraceptive for a period of 2 years among healthy women was not associated with clinically significant changes in fasting plasma glucose or insulin levels (54). Only 2% of the women on norgestimate-containing COC developed abnormal fasting glucose levels after six months of use, while 35% lowered their initial abnormal glucose levels into the normal range after six months of use (71). Additionally, norgestimate containing COC have also shown favorable effect on lipid profile, including elevation of HDL, reduction of LDL and improved HDL/LDL ratio (69;71). These results suggest that norgestimate containing COC show minimal effect on glucose-insulin dynamics.

No differential effect on insulin clearance was also observed between African-American obese (n=7) vs. non African-American obese (n=7) women after six months of COC use. It is

interesting to note that even in a small sample size of 7 in each group; the effect reached a significance level of 0.0957, suggesting that it may be worthwhile to repeat this study in a larger cohort.

Changes in insulin clearance with six months of COC use was found to have a negative relationship with changes in AIRg and a positive relationship with changes in insulin sensitivity, as measured by the minimal model. Causations cannot be established with this study. Limited information exists regarding the association between hyperinsulinemia and decreased insulin clearance. It is also possible that with a higher AIRg, pancreatic insulin secretion may continue while insulin is being cleared, resulting in a net reduced insulin clearance.

In the present study, total insulin clearance was quantified from insulin concentration obtained during FSIVGTT test. It is reported that the plasma insulin concentration declines following intravenous insulin infusion, with at least two exponential decay (63). Hence, steady state insulin concentration was used to determine the total insulin clearance. Total clearance was obtained by the product of insulin elimination constant and volume of distribution (Vd= Dose/C_0), with dose of insulin infusion adjusted to body weight of each individual. However, some methodological inadequacies are present in this study. Insulin degradation is described as linear first order kinetics, considering the narrow concentration intervals. This could be a possible limitation of the study as some amount of non-linearity has been demonstrated owing to saturable processes (73). The given study does not provide a measure of endogenous insulin production and its subsequent clearance. In the case of exogenous insulin infusion, endogenous

insulin secretion is suppressed by somatostatin (29). Under this condition, insulin clearance by the liver and peripheral tissue occurs in parallel. Metabolic clearance (MCR) of endogenous and exogenous insulin is related by the following formula: (29)

$MCR_{endogenous} = MCR_{exogenous} / (1-E_h)$;

Where E_h is the hepatic extraction ratio

Besides methodological constraints, any discrepancy in the insulin clearance data from the proposed hypothesis could also be due to the small number of women studied in each of the groups. Lack of significant differences could also be attributed to lack of power, owing to small sample size. Also, the study was conducted for a period of six months. Considering that contraception methods are used over a long period of time for their desired purpose, it may be more meaningful to study changes over a period longer than six months. It has been hypothesized that an inverse relationship exist between dietary carbohydrate to fat ratio and insulin clearance (36). Hence, type of diet could have an effect on insulin clearance, which was not controlled for in this study. It will be impractical to control study participants' diet for the duration of the study.

The purported study is significant as it provided information on total insulin clearance in lean vs. obese women after six months of COC use, and addressed another potential mechanism of

hyperinsulinemia in COC users. The study also provided insights on the effect of insulin clearance among African-American obese vs. non African-American obese women.

The choice of COC formulation is important as excessive estrogenic activity is known to cause untoward thromboembolic effects, while progestins are known to have a deleterious effect on carbohydrate metabolism. Overall, the pilot study showed no differential effect on insulin clearance in lean vs. obese women after six months of COC use [Ortho Tri Cyclen; containing ethinyl estradiol 35mcg and norgestimate0.18/ 0.215/0.25 mg]. The study may suggest that norgestimate has minimal effect on insulin clearance, in contrast to previous studies using other progestins.

Given that insulin clearance does not seem to be affected by the COC used in this study, future work on hyperinsulinemia as a result of contemporary COC use should focus on mechanisms for increased pancreatic insulin secretion and/or insulin resistance, and the effects of exogenous hormones on these processes.

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